Helping Gail w/ subcloning

Looked at the cells.

(a) Yesterday's transfections were made in marvelous accuracy. They are nearly confluent & look great. The only exception is plates 2A & 2B. They show toxicity — probably due to the high lipofectin concentration. Time of lipofection may also be an issue.

(b) P3A & P2A cells look good. ready to pass Monday.

Gail appears to have CMV-gal in pAE1 or 1A. To confirm
a) grow up clone
b) make restriction sites
c) transfect LNCaP for β-gal activity.

Pass cells; set-up new plates
20 x 6 cm plates for tomorrow
9 x 150 cm boxes for freezing down
3 x 100 cm boxes for carrying

Gail is making mini's and doing restriction digests. SL has 6 BHall clones for HindIII, 22 clones of 6kb HindIII PSC in CN70 (β-gal in BS).

→ Make 10 ml of 10x ligase (without ATP) per protocol in computer - record of 10x I made at beginning of project. Sterile filtered. Freeze ~~10 ml~~ 1 ml aliquots

5.0 ml 1M Tris (7.6)
1.0 ml 1M MgCl2
1.0 ml 0.5M DTT
50 µl 10 mg/ml BSA
2.95 ml H2O

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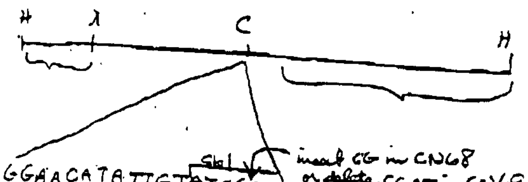
Recorded by D. Hamilton

CN70 = β-gal in BS
CN71 = CMV HSV tk

cloned

PSE
5800bp



GGAACATATTGTATCGAT — insert CG in CN68 or delete CGAT in CN69
GRE (ARE probable)
13 of 15 bp match for GRE

AGENDA

I. Characterization of PSE - Eric, Lena, Joe
5' end and 3' end of PSE defined within 200 bp
ARE within the Cla I site required
Sequencing
Thus, at least three elements: ARE, Left and Right within PSE

Movement and orientation of minimal PSE - Lena (4 of 4)
Are these tested in LnCaP yet?
Joe has sequenced my (CN42 Cla I Klenow) CN68
Gel Shift with 200 bp segments across PSE - Eric
fragment which spans the 5' end of the XbaI site binds protein from nuclear extract of LNCap

II. Transgenic Mice Constructs - Gail, Dan
Gail 1. 6kb driving β-gal (need 6 kb in CN70, β-gal in BS) *(sent today, ready for injection)*
Lena 2. minimal enhancer driving β-gal
we have it 3. **6kb driving DT-A (CN45)** *produced a male mouse*

III. Adenovirus Constructs - Gail, Lena, Dan
1. PSE minimal enhancer driving β-gal
2. PSE minimal enhancer driving CAT
3. PSE minimal enhancer driving DT-A

4. CMV driving HSV-tk *done*
5. CMV driving cytosine deaminase } *for competent V, if we add drug we can kill virus*
6. CMV driving β-gal *positive control*

ΔE1 & BHG give back virus when co-transfected & gives recombinant in cells.

Construction of Adenovirus vector
ΔE1sp1A, ΔE1sp1B, BHG10, BHG11, BHGE3, FG140, pXC1, pABS.4

IV. Tissue Specificity of Minimal Enhancer - InVitro - Eric and Joe
Results *good result of last week being repeated this week*

V. Tissue Specificity of PSE - InVivo - Eric, Joe, Lena

Direct Injection of DNA into Tumors *1 exp neg too big exp*
Technique of Intra-tumor injection *1 exp ongoing*
CAT Assay

VI. Liposomes - Henry

Experiments

~~Competent cells~~ Commercial (Life) vs Lena lower vs Dan lower
→ Use commercial cells for next ligations

CN68 doesn't alter site as much as 5' of CN69. These changes don't change GRE region but rather the flanking region but still affect expression of CAT. There is less activity with CN69 than CN68 as expected

4'5 Cory Gorman from Megabios talking about liposomes
Henry in Tucson Mon. & Tues.
Check lunch schedule of Conf. room for virus lesson
Check ³H chloramphenicol - Delivery today! (Friday lunch)
Q for Eric: IPA salt out. For removal of EtBr. from CsCl/EtBr/DNA from gradient

Water solvates IPA - if water is high salt & IPA is hi salt Water cannot solvate IPA & you get an interface

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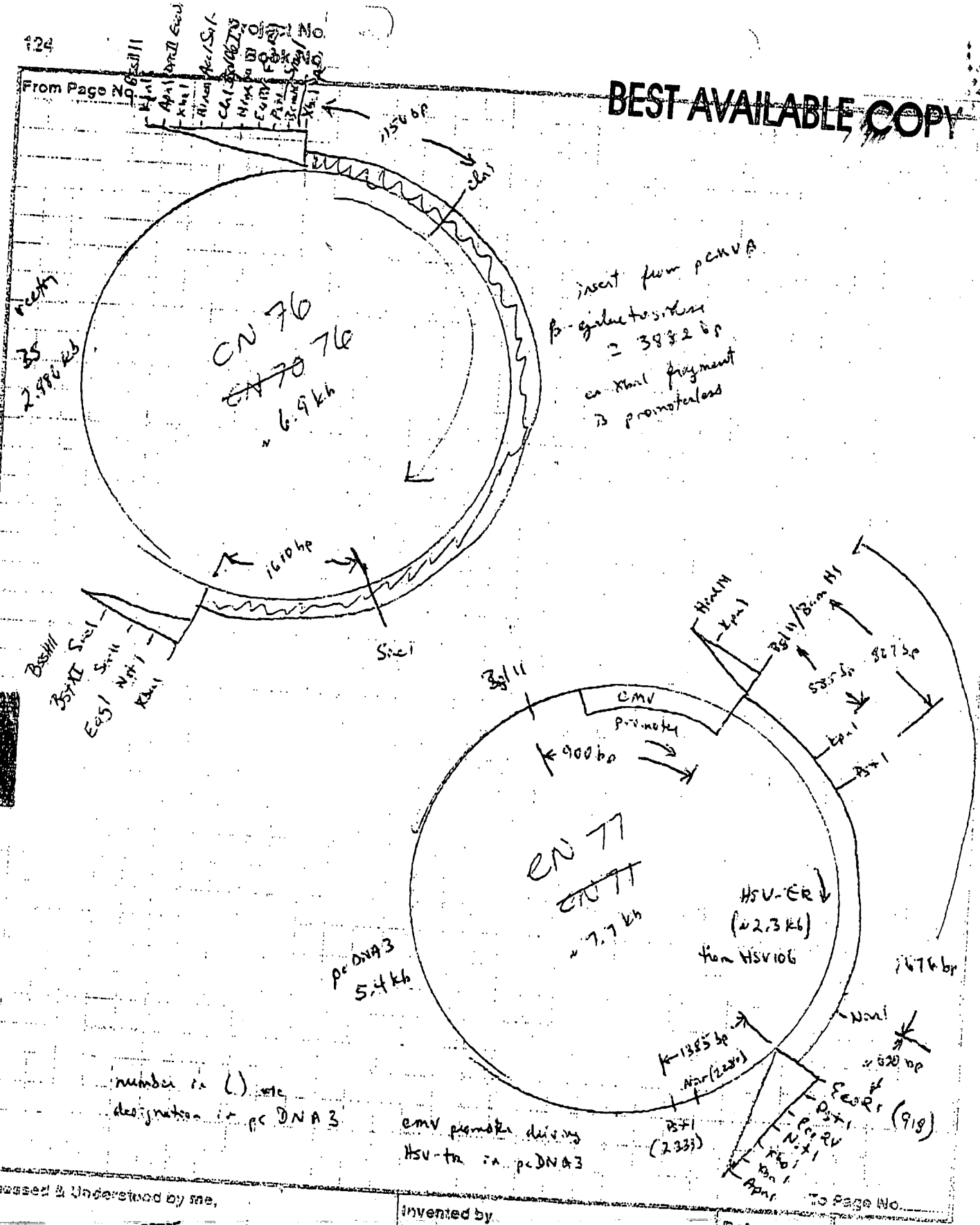

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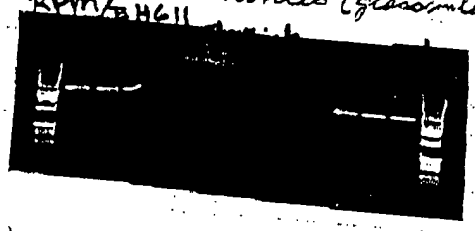
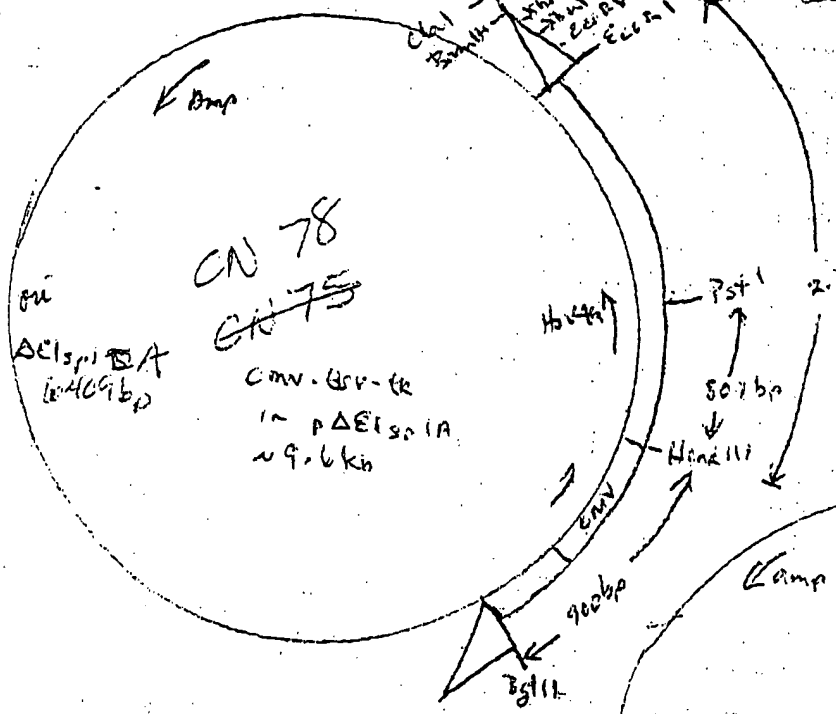
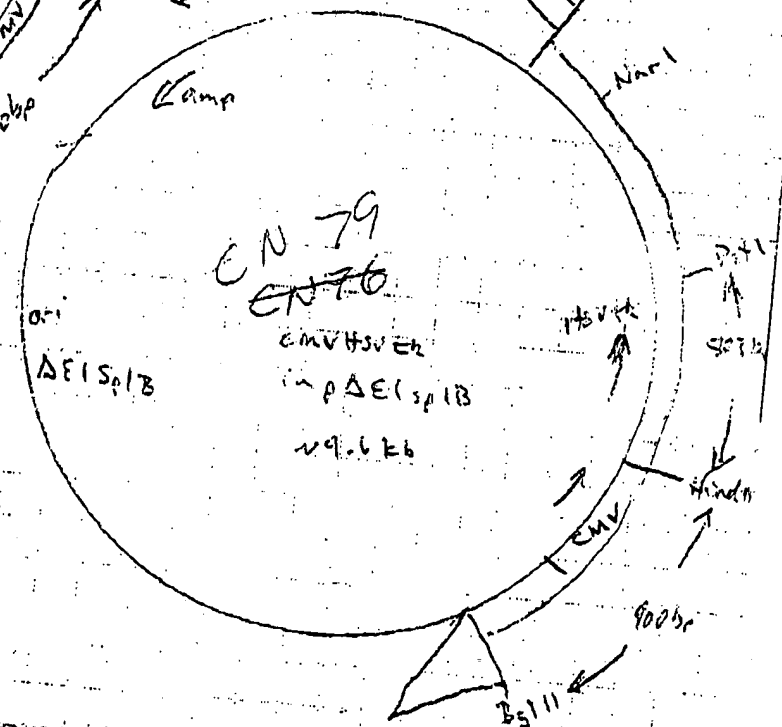
Dan has grown BHG 11 another A, B, C cultures to HindIII screen.

I am mini prepping 1.5 ml of
8 µl DNA
1 µl #2 10× Buffer
1 µl HindIII ≈ 3 µl — No luck

I called Bio 101 with regard to the problems I'm having with very large plasmids in their RPM kit. They are sending new matrices (glassmilk)







Both CN 78 & CN 79 are
BglII + EcoRI of CN 71
into EcoRI + BglII of
ΔEI sp IA and ΔEI sp IB
respectively

Insert ≈ 3.2 kb
Vector = 6.4 kb

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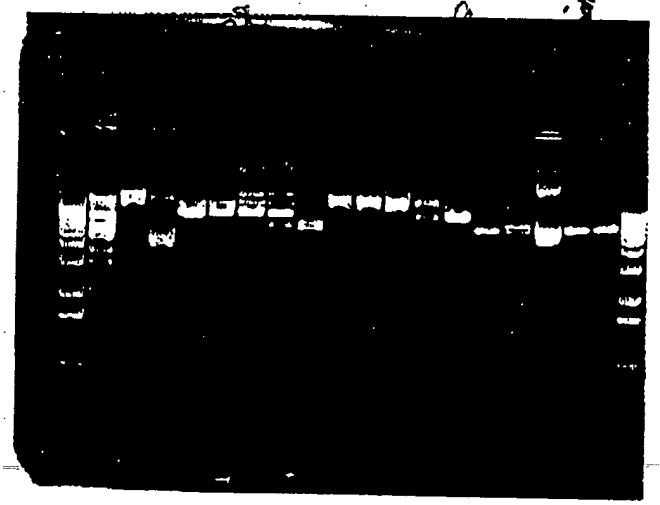
Concentrations	OD260	OD280	260/280	Conc	Yield
FG140 16.122	0.076	0.036	2.1	380 µg/ml	152 µg
CN 78-1 16.122	0.015	0.002	7.5	75 µg/ml	15 µg
CN 78-2 16.122	0.437	0.208	2.1	2.2 mg/ml	437 µg
CN 79-1 16.122	0.022	0.012	1.8	110 µg/ml	22 µg
CN 79-2 16.122	0.264	0.133	2	1.3 mg/ml	264 µg

CN 78 looks fine: 1K cut of HindIII, EcoRI, BglII, EcoRI + BglII gives insert and HindIII cuts the insert into 2.3 kb & 900 bp as it should

CN 79 may be fine but: it appears NotI and HindIII (lanes 10 & 11) were omitted: i.e. no cut, but EcoRI cuts 1X, BglII cuts 1X, EcoRI + BglII gives the 3.2 kb insert, and HindIII cuts the insert into 2-3 (visible in photo) & 900 (visible by eye but not in photo) as it should



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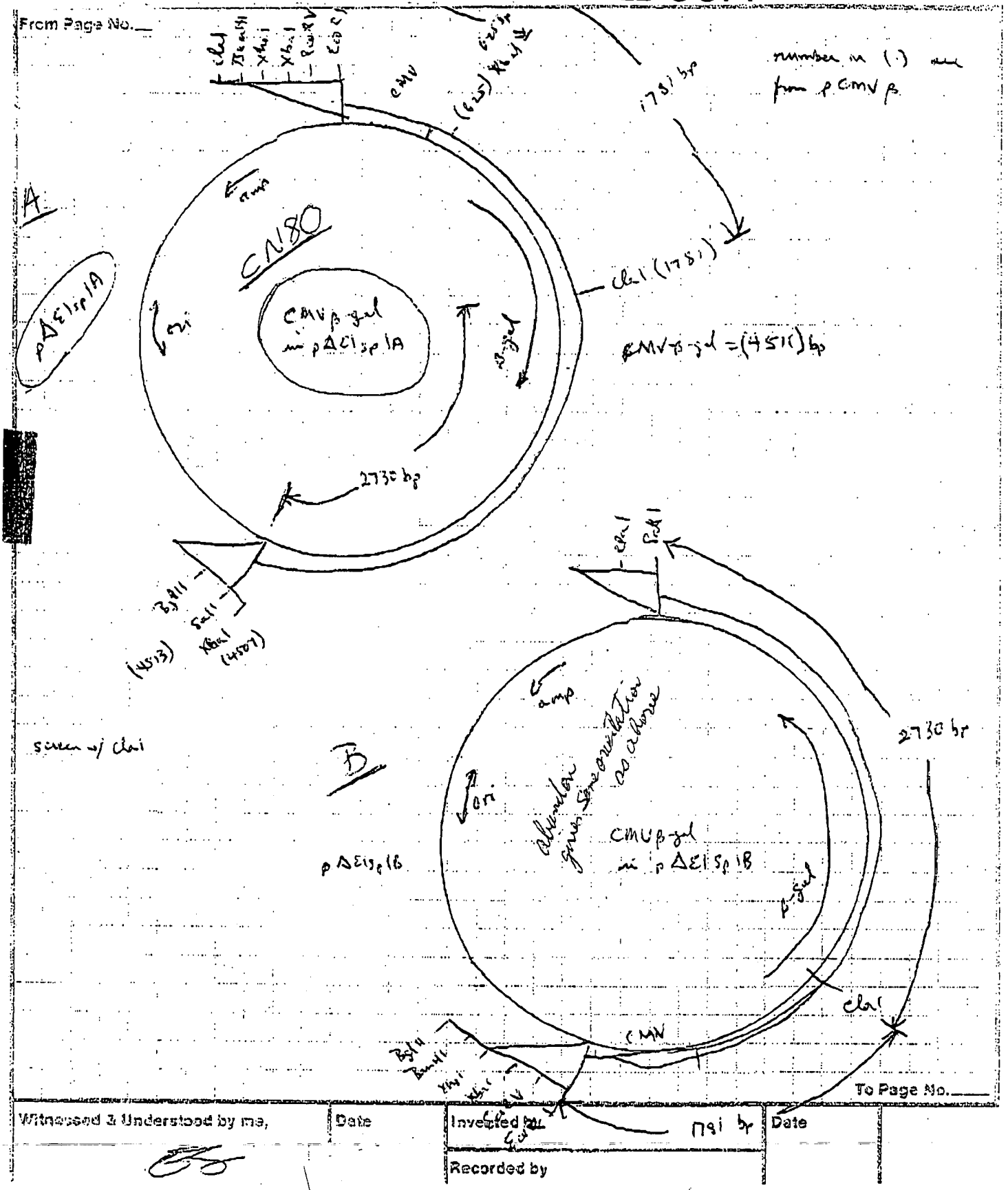

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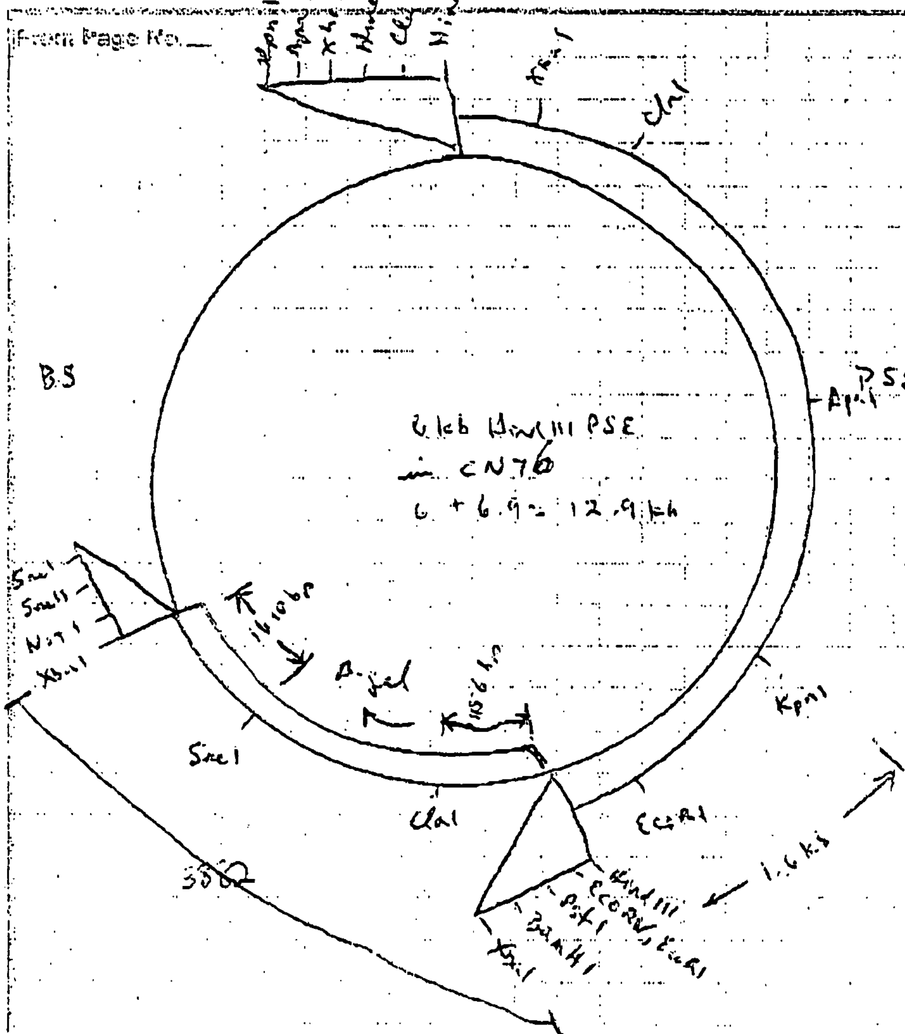

XbaI + ~~3.5~~, 3.9, ~~2.9~~ 13.4
− 0.5, 3.9, ~~2.9~~ 8.4
ClaI + 1.8, ~~2.7, 6.4~~ 5.4, 5.6
− 3, 4.2, 5.6
ApaI + 3.2, 9.7
KpnI + 4.4, 8.5
− 1.6, 11.3

PSE (~6 kb)

AmpIII − ~~6800~~ + 6782

screen w/ KpnI

if correct orientation should get ~ 4.4 kb
if opposite orientation should get ~ 1.6 kb

9882
6782

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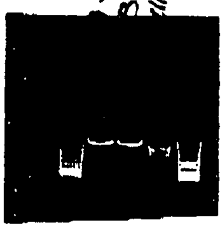
Dan picked more 22 2ml cultures of lig 16.121.3 this weekend. Of the 6 cultures I minied this weekend 1 was correct but wrong orientation ∴ there is a chance we will get something out of these 22.

Mini RPM & KpnI cut
4 µl dH_2O
1 µl 10x BSA
1 µl 10x NEB1
1 µl KpnI 20/µl
3 µl DNA

Mini RPM. 6 TB amp cultures of BHG11 - 2 dif plates 1 commercial DNA 1 - from previously good mini. These were picked on Sat & expanded to 10ml Sun eve. Dan collected 1½ ml for minis & fed ea culture 10 ml TBamp.

16.123 prep
2µl



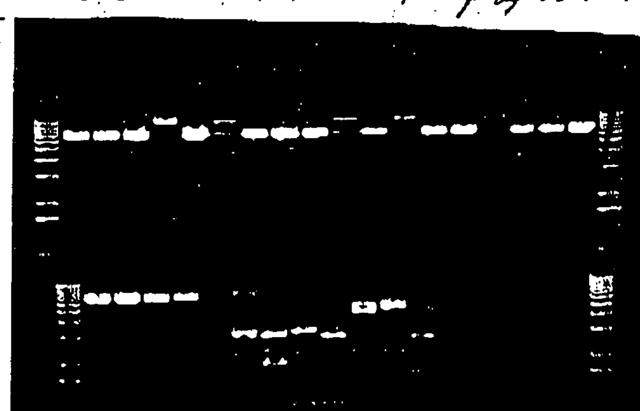
Conc gel Vectors & Inserts 16.123

CMV CH-I III prep ##B look equal to ~1/15 of 1µg ladder
1µg/15 = 0.066... 0.067/2µl = 33 ng/µl

Dan did RE digest of SV CAT 16.78 see book 15.123 this prep look good

OD 260 0.550
OD 280 0.321
260/280 1.7
Conc = 2.8 mg/ml

Dan [illegible] [illegible] digests of 16.126.3 above clones 3, 5, 6 shows small band at ~4.4 but it is very light I don't know whether to believe it or not. I will do more cuts tomorrow



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